

CLAIMS

1. A method for detecting and quantitating a microorganism having a specific function and its gene from the 5 natural environment, the method comprising the steps of:

1) subjecting a microorganism-containing sample collected from the natural environment to serial dilution, then incubating it under the conditions under which the microorganism having a specific function can grow, and then 10 counting the grown microorganism having a specific function, and in parallel with these operations, counting the total number of microorganisms in the above microorganism-containing samples, and simultaneously counting the total number of heterotrophic microorganisms, and estimating the 15 dominant level of the microorganism having a specific function in the natural environment from the ratio of the number of the microorganism having a specific function to the total number of microorganisms and/or of heterotrophic microorganisms;

2) extracting DNA from the microorganism in a liquid 20 culture broth of the highest dilution ratio at which the growth of the microorganism is judged as positive, and amplifying specific gene domains using said DNA as templates, followed by cloning;

25 3) examining the difference of the gene domains thus cloned, and determining the nucleotide sequences thereof; and

4) identifying the microorganism having a specific function inhabiting the natural environment from the nucleotide sequences data thus determined.

2. A method as claimed in Claim 1, wherein the number 5 of the microorganism having a specific function and the total number of heterotrophic microorganisms is counted by an MPN method, the total number of the microorganisms is counted by a direct microscopic counting method, and the growth of the microorganism having a specific function is judged by 10 observation under a microscope.

3. A method as claimed in Claim 1, wherein the microorganism having a specific function is a microorganism which degrades a specific chemical substance.

4. A method as claimed in Claim 2, wherein the 15 microorganism having a specific function is a microorganism which degrades a specific chemical substance.

5. A method as claimed in Claim 3, wherein the specific chemical substance is a harmful chemical substance.

6. A method as claimed in Claim 4, wherein the specific 20 chemical substance is a harmful chemical substance.

7. A method as claimed in Claim 3, wherein the specific chemical substance includes petroleum and petroleum components.

8. A method as claimed in Claim 4, wherein the specific 25 chemical substance includes petroleum and petroleum

components.

9. A method for assessing the function of a microbial population in the natural environment by analyzing succession of the microorganism existing predominantly in the natural environment using the method as claimed in Claim 1.

10. A method for assessing the function of a microbial population in the natural environment by analyzing succession of the microorganism existing predominantly in the natural environment using the method as claimed in Claim 2.

10 11. A method for analyzing and assessing a polluted environment using the method as claimed in Claim 1.

12. A method for analyzing and assessing a polluted environment using the method as claimed in Claim 2.

13. A method for analyzing and evaluating an environment 15 polluted by harmful chemicals using the method as claimed in Claim 3.

14. A method for analyzing and evaluating an environment polluted by harmful chemicals using the method as claimed in Claim 4.

20 15. A method for analyzing and evaluating an environment polluted by harmful chemicals using the method as claimed in Claim 5.

16. A method for analyzing and evaluating an environment 25 polluted by harmful chemicals using the method as claimed in Claim 6.

17. A method for analyzing and evaluating an oil-polluted environment using the method as claimed in Claim 7.

18. A method for analyzing and evaluating an oil-polluted environment using the method as claimed in Claim 8.

5 19. A method as claimed in Claim 1, wherein the microorganism having a specific function is a microorganism producing a useful enzyme.

10 20. A method as claimed in Claim 2, wherein the microorganism having a specific function is a microorganism producing a useful enzyme.

21. A 16S rDNA having the nucleotide sequence represented by any of SEQ ID NOS: 1 to 4.

22. An RNA or DNA probe with the length of from 10 to 50 bases which has a part of the nucleotide sequence represented by any of SEQ ID NOS: 1 to 4 and is hybridizable specifically with a petroleum-degrading bacterium belonging to the genus *Cycloclasticus*.

23. An RNA or DNA probe as claimed in Claim 22, wherein the part of the nucleotide sequence represented by any of SEQ ID NOS: 1 to 4 is selected from the group consisting of the nucleotide sequences represented by SEQ ID NOS: 5, 6 and 7.

24. An RNA or DNA probe as claimed in Claim 22, which is used in detection or quantification of a petroleum-degrading bacterium belonging to the genus *Cycloclasticus*.

25 25. An RNA or DNA probe as claimed in Claim 23, which

is used in detection or quantification of a petroleum-degrading bacterium belonging to the genus *Cycloclasticus*.

26. An RNA or DNA probe as claimed in Claim 24, wherein the petroleum-degrading bacterium belonging to the genus 5 *Cycloclasticus* is *Cycloclasticus pugetii* or its closely related species.

27. An RNA or DNA probe as claimed in Claim 25, wherein the petroleum-degrading bacterium belonging to the genus 10 *Cycloclasticus* is *Cycloclasticus pugetii* or its closely related species.

28. An RNA or DNA probe as claimed in Claim 22, which is used in screening a petroleum-degrading bacterium belonging to the genus *Cycloclasticus*.

29. An RNA or DNA probe as claimed in Claim 23, which 15 is used in screening a petroleum-degrading bacterium belonging to the genus *Cycloclasticus*.

30. An RNA or DNA probe as claimed in Claim 28, wherein the petroleum-degrading bacterium belonging to the genus 20 *Cycloclasticus* is *Cycloclasticus pugetii* or its closely related species.

31. An RNA or DNA probe as claimed in Claim 29, wherein the petroleum-degrading bacterium belonging to the genus *Cycloclasticus* is *Cycloclasticus pugetii* or its closely related species.

25 32. A method for detecting and quantitating a

petroleum-degrading bacterium belonging to the genus *Cycloclasticus* using the RNA or DNA probe as claimed in Claim 22.

33. A method for detecting and quantitating a
5 petroleum-degrading bacterium belonging to the genus *Cycloclasticus* using the RNA or DNA probe as claimed in Claim 23.

34. A method as claimed in Claim 32, wherein the
petroleum-degrading bacterium belonging to the genus
10 *Cycloclasticus* is *Cycloclasticus pugetii* or its closely
related species.

35. A method as claimed in Claim 33, wherein the
petroleum-degrading bacterium belonging to the genus *Cycloclasticus* is *Cycloclasticus pugetii* or its closely
15 related species.

36. A method for screening a petroleum-degrading
bacterium belonging to the genus *Cycloclasticus* using the RNA
or DNA probe as claimed in Claim 22.

37. A method for screening a petroleum-degrading
20 bacterium belonging to the genus *Cycloclasticus* using the RNA
or DNA probe as claimed in Claim 23.

38. A method as claimed in Claim 36, wherein the
petroleum-degrading bacterium belonging to the genus *Cycloclasticus* is *Cycloclasticus pugetii* or its closely
25 related species.

39. A method as claimed in Claim 37, wherein the petroleum-degrading bacterium belonging to the genus *Cycloclasticus* is *Cycloclasticus pugetii* or its closely related species.

5 40. A method for identifying a petroleum-degrading bacterium belonging to the genus *Cycloclasticus* by means of DNA/DNA or DNA/RNA hybridization using an RNA or DNA probe homologous to SEQ ID NO: 1 or as claimed in Claim 22.

10 41. A method for identifying a petroleum-degrading bacterium belonging to the genus *Cycloclasticus* by means of DNA/DNA or DNA/RNA hybridization using an RNA or DNA probe homologous to SEQ ID NO: 1 or as claimed in Claim 23.

15 42. A method as claimed in Claim 40, wherein the petroleum-degrading bacterium belonging to the genus *Cycloclasticus* is *Cycloclasticus pugetii* or its closely related species.

20 43. A method as claimed in Claim 41, wherein the petroleum-degrading bacterium belonging to the genus *Cycloclasticus* is *Cycloclasticus pugetii* or its closely related species.